An evaluation of an InPouch™ TV culture method for diagnosing *Trichomonas vaginalis* infection

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Abstract

A new culture method for *Trichomonas vaginalis*, the InPouch™ TV test, was evaluated for its sensitivity and specificity in supporting growth of trichomonads. Five clinical isolates remained viable for periods from 41 to 131 days. A strain from the ATTC 30001 remained viable for 91 days. As few as four trichomonads/ml of culture medium could be viewed microscopically within 24 h. Doubling time for growth of trichomonad varied between 5 to 8 h. In a clinical study of 102 wet-mount negative specimens, 15 culture positive patients were observed with the InPouch™ TV test compared with 12 of the same patients with Hollander's fluid medium.

Introduction

The protozoan parasite *Trichomonas vaginalis* is known to cause one of the most common forms of sexually transmitted disease in the world.[1] A diagnosis of this infection only on a clinical basis, such as characteristics of a vaginal discharge, may be erroneous.[2] Various laboratory methods have been employed for the detection of *T vaginalis* in vaginal discharges: the saline wet mount; different stains and smears including Giemsa, Gram, Papanicolaou and acridine orange; enzyme immunoassay (EIA); monoclonal antibody staining of direct specimens; and the latex slide agglutination test.[3–6] These methods, when compared with isolation of the organism by culture, vary widely in sensitivity and specificity.[4 7 8] Some non-cultural techniques require expensive equipment and performance times longer than normal patient contact. Isolation of *T vaginalis* is regarded by some as too expensive, time consuming and difficult to be of routine value.[9]

This study describes an evaluation of the InPouch™ TV test (BioMed Diagnostics Inc, Santa Clara, USA) which is a disposable culture system for the maintenance, transport, and detection of *T vaginalis* in clinical specimens.

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Materials amd methods

Description of the InPouch™ TV test

The InPouch™ TV test is both a transport and culture system. It is constructed of a clear plastic film which eliminates medium loss and maintains a reduced Eh. Each pouch is divided into two chambers which are separated by a channel that allows the medium to pass between them (fig 1). The lower chamber contains 4 ml of a selective medium that is inhibitory for both yeast and bacteria.

A small volume of medium is introduced into the upper chamber by applying pressure on the bottom chamber. A wire tape attached to the upper chamber is used to open the pouch. The specimen obtained on a cotton-tipped applicator stick is mixed with the medium in the upper chamber. Before re-introduc-

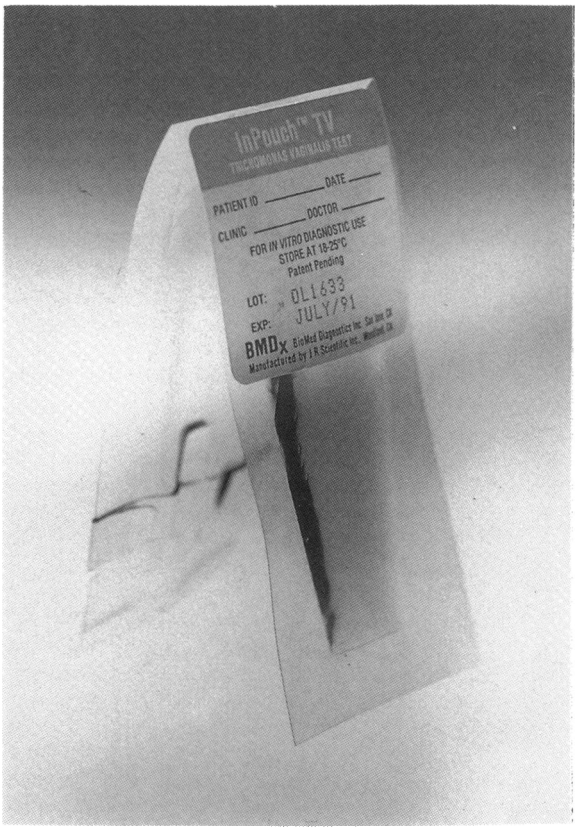

Fig 1 Two-chambered plastic pouch.

ing the specimen into the medium in the bottom chamber, it may be examined microscopically under low power ($\times 10$) for presence of trichomonads. Before examination it is best to incubate the pouch vertically at 37°C for 30 min. This procedure substitutes for the saline wet mount. Then the mixture is squeezed into the bottom chamber by rolling down and sealing the top chamber.

After incubation at 37°C for 24 h, the bottom and side seams of the pouch are vigorously massaged. This releases sequestered trichomonads into the medium. A plastic viewer (fig 2) is placed over the bottom of the pouch before microscopic evaluation. This allows the pouch to be placed on the microscope stage and immobilises the medium for easier evaluation. The test is read under low power scanning of the entire open window of the viewer for approximately 2 min, and when necessary high power (§40) may be used to confirm the diagnosis. A negative specimen should be re-incubated and examined at 48 h and 5 days.

Laboratory methods

Viability studies were performed in the InPouch™ TV test using five clinical isolates and an American Type culture Collection (ATTC) strain 30001.

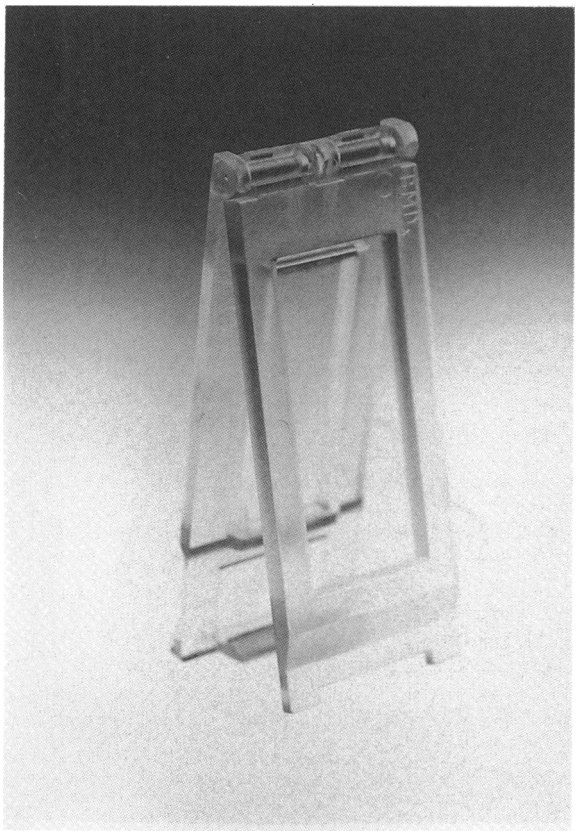

Fig 2 Plastic receiver.

Specimens were evaluated microscopically at weekly intervals for presence of motile forms. At appropriate periods subcultures were performed to demonstrate reproducibility of the trichomonads.

Stability at room temperature storage was determined on a monthly basis by comparing doubling reproductive time of trichomonads in the same lot number of pouches. A 30 μl inoculum of trichomonads was obtained from a culture that demonstrated a viable count between $1{\cdot}3 \times 10^4$ and $5{\cdot}0 \times 10^5$. Trichomonad density was determined using a haemocytometer counting chamber.

An experiment was carried out to determine the sensitivity of the InPouch™ TV test using the ATCC 30001 strain of trichomonads. The culture was diluted so that the final concentration of organisms represented 400, 40 and 4/ml of medium within the pouch.

Clinical study

During 3 months in 1989, 134 women with vaginal discharge were examined in two Sexually Transmitted Disease Clinics of the Contra Costa County Public Health Department for *T vaginalis*. Specimens were examined by saline wet mount and by culture. The latter consisted of a conventional Hollander fluid (HF)[10] medium and the InPouch™ TV test. Only wet mount negative specimens were cultured for *T vaginalis*.

Results

Viability studies demonstrated trichomonad growth between 41 and 132 days (table 1). These data represent the longest growth periods for the strains and does not indicate similar duplication after each

Table 1 Viability studies demonstrating extended growth in the InPouch™ TV test

Clinical isolates	*Number of days viable*
TV 690	41
TV WL-1	44
TV WL	63
TV 3	110
TV 2	132
ATCC 30001	98

Table 2 Stability of the InPouch™ TV test medium at room temperature storage

TV strain	*Initial count before dilution of 30 µl*	*24 h count*	*48 h count*
ATTC 30001	$7{\cdot}2 \times 10^4$	$4{\cdot}7 \times 10^3$	$3{\cdot}9 \times 10^4$
ATTC 30001	$3{\cdot}9 \times 10^5$	$7{\cdot}5 \times 10^3$	$5{\cdot}8 \times 10^4$
ATTC 30001	$3{\cdot}5 \times 10^5$	$2{\cdot}3 \times 10^4$	$1{\cdot}1 \times 10^5$
TV WL	$1{\cdot}3 \times 10^4$	$1{\cdot}4 \times 10^4$	$7{\cdot}1 \times 10^5$
ATTC 30001	$3{\cdot}1 \times 10^5$	$2{\cdot}3 \times 10^4$	$3{\cdot}3 \times 10^5$
ATTC 30001	$4{\cdot}3 \times 10^5$	$9{\cdot}3 \times 10^4$	$4{\cdot}9 \times 10^5$

Table 3 Sensitivity of the InPouch™ TV test employing the ATCC strain 30001

	Hours/Days				
Number of organisms	*24 h*	*48 h*	*72 h*	*96 h*	*7 days*
400/ml	$4{\cdot}1 \times 10^3$	$1{\cdot}9 \times 10^4$	$7{\cdot}2 \times 10^4$	$4{\cdot}5 \times 10^5$	$5{\cdot}7 \times 10^3$(V) $1{\cdot}1 \times 10^6$(T)
40/ml	NC*	$4{\cdot}1 \times 10^3$	$2{\cdot}7 \times 10^4$	$2{\cdot}4 \times 10^5$	$6{\cdot}1 \times 10^3$(V) $1{\cdot}2 \times 10^6$(T)
4/ml	NC†	NC†	$5{\cdot}0 \times 10^3$	$1{\cdot}6 \times 10^4$	$3{\cdot}1 \times 10^5$(V) $3{\cdot}3 \times 10^5$(T)

V, viable trichomonads counted; T, total number of trichomonads counted.
*Counts less than 1×10^3 but motile organisms observed in 3 tests.
†Counts less than 1×10^3 but motile organisms observed in 2 tests.
These counts represent an average of 3 tests.

Table 4 Trichomonas vaginalis cultured from wet mount negative patients

Number of specimen tested	*Number of wet mount tests*		*Number of wet mount negative-culture positive tests*	
	Positive	*Negative*	*InPouch™ TV*	*HF medium*
134	32	102	15	12

subculture. Most trichomonad subcultures in the InPouch™ TV test demonstrate viability for periods in excess of 21 days.

Stability of the medium at room temperature has been confirmed for a period of 6 months by determining doubling reproductive times of the trichomonads in the pouch (table 2). Trichomonad doubling time as recorded on a monthly basis has varied between 5 and 8 h depending on the strain cultured.

Trichomonad growth in the InPouch™ TV test over a 7 day period is shown in table 3. In all pouches, except one with an inoculum of trichomonads 4/ml, organisms could be observed either microscopically at 24 h or counted (400/ml). The absence of visible trichomonads in one test and the resulting density at 72 h may indicate an inoculum of less than 4/ml trichomonads. Viable organisms at 96 h were representative of the original inoculum density. At 7 days an inoculum of 4/ml trichomonads produced the highest density ($3{\cdot}1 \times 10^5$), whereas 400/ml had the lowest ($5{\cdot}7 \times 10^3$) because of depletion of nutrient component.

T vaginalis was isolated by the saline wet mount in 32 of the 134 patients, 23·8% (table 4). In the 102 wet mount negative specimens an additional 15 patients were positive for *T vaginalis* (14·7%). Fifteen of the culture positive patients were observed by the InPouch™ TV test, whereas 12 of these were positive with HF medium. The wet mount sensitivity was 68%. When compared with the InPouch™ TV test the sensitivity to the HF medium was 80%.

Discussion

The InPouch™ TV test for *T vaginalis* offers many unique advantages when compared with other contemporary in vitro laboratory diagnostic tests. Its 6 month stability at room temperature offers significant savings in media. The pouch has the versatility of being used both for specimen transport and culture. Specimens may be mailed and maintain trichomonad viability for approximately 1 week. The medium is selective with effective anti-bacterial and anti-fungal activity. Since only a microscope and viewer are required for reading the test, it eliminates slide preparation and saves technical time.

Trichomonads have demonstrated growth in the medium with less than 10/ml organisms. Because the pouch is so simple to view microscopically, positive tests can be observed with counts of less than 10/ml trichomonads.

The most significant advantage of the InPouch™ TV test is its clinical efficacy. Its sensitivity and specificity were superior when compared with HF medium.

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